USE OF 9-AMINOACRIDINE AS A PROBE IN A KINETIC STUDY OF DNA-POLY-L-LYSINE INTERACTION

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SUMMARY: An attempt has been made to use a fluorescent dye as a probe in a kinetic study of DNA-poly-L-lysine interaction by the stopped-flow method. It is found that 9-aminoacridine is suitable for this purpose. The results have indicated that polylysine binds to DNA at least in two steps; a slow unimolecular process ($\tau \sim 2$ msec) being preceded by a rapid bimolecular one ($\tau << 1$ msec). The usefulness of this method for the kinetic study of the interaction between DNA and basic polypeptides has been emphasized.

Histone is known to inhibit the synthesis of DNA in vitro to a substantial extent (1). The nature of the interaction between DNA and histone may be among subjects of prime importance in understanding the mechanism of genetic control in vivo. Some structural information of the interaction has been obtained with various methods including X-ray analysis, often with basic polypeptides such as polylysine and polyarginine as a model for histone (2, 3). It has been suggested that the basic polypeptides twist along a groove of DNA through one-to-one electrostatic interaction between the negatively charged phosphate group of DNA and the positively charged amino group of the basic polypeptides. No kinetic study, however, has been made on the DNA-basic polypeptide interaction,

although it is important from the biological viewpoint.

It is difficult to observe directly the time course of the binding process between DNA and basic polypeptides through the small absorbance change in ultra-violet region. However, since the binding of some cationic dyes to DNA is thought to be competitive with that of basic polypeptides (4), such a dye may be used as a "probe", or an indicator which tells us whether or not basic polypeptide is tightly bound to DNA. By using a fluorescent dye as a probe, we have been able to observe directly the time course of DNA-polylysine interaction with the fluorescence stopped-flow technique. In this paper, it will be shown that a fluorescent dye 9-aminoacridine can be a useful probe for the kinetic study of the interaction between DNA and the basic polypeptides.

EXPERIMENTAL

Solutions of 9-aminoacridine (Merck), salmon sperm DNA (Sigma), and poly-L-lysine (Protein Research Foundation, Osaka) were prepared in 1 mM phosphate buffer of pH 6.9, the ionic strength being approximately 0.001. The average degree of polymerization of polylysine used was estimated to be ca. 100 from CM-cellulose column chromatography (5). The static fluorescence and absorption spectra were measured at about 20°C with a spectrophotometer (Hitachi EPS-3T), equipped with a fluorescence attachment (Hitachi G-3). Stopped-flow measurements were done at 25°C with a stopped-flow apparatus (Union Giken Co., Ltd. SF-70) in the following manner: The reaction was commenced by rapidly mixing equal volumes of DNA-dye mixture and polylysine solutions, and the change in fluorescence intensity (excited at 400 nm) was recorded on a storage oscilloscope screen. The fluorescence emission was observed from the right angle to the excitation beam through a cut-off filter

(Toshiba VY43) transmitting above 430 nm. A cylindrical quartz observation cell of 2 mm diameter was used with 5 mm vertical slit length. The dead-time of the apparatus was about 1 msec. The time constant of the electronic circuit for detection was well below 0.2 msec.

RESULTS AND DISCUSSION

The fluorescence of 9-aminoacridine is quenched when bound to DNA (6). However, when an excess amount of polylysine (P/L = 1/3, where P/L denotes the molar ratio of DNA phosphate to lysine residue) was added to DNA-dye mixture, the fluorescence was completely recovered to the level of free dye, whereas the extent of recovery was small with lysine monomers even at P/L = 1/120. The change in fluorescence intensity due to the possible interaction of the dye and polylysine itself was negligible. Therefore, the fluorescence increase observed by the stopped-flow method is attributable to the process in which the dye is expelled from DNA by polylysine.

The time course of the change in fluorescence intensity (oscilloscope trace) shown in Figure 1 obeyed an apparent first order kinetics giving a single relaxation time τ. Figure 2 shows the plot of the reciprocal relaxation time $1/\tau$ against the P/D ratio (the molar ratio of DNA phosphate to dye) at a fixed dye concentration (5×10^{-5} M) and a fixed P/L ratio (1/3). This figure also shows the relationship between $1/\tau$ and the sum of the concentrations of DNA phosphate and lysine residue $(C_P) + (C_L)$. If the reaction proceeded in a simple bimolecular process, $1/\tau$ should increase with $(C_P) + (C_L)$ (7). The saturation behavior of $1/\tau$ seen in Figure 2, however, clearly shows that it is not the case, but is consistent with a sequential mechanism in which a rapid bimolec-

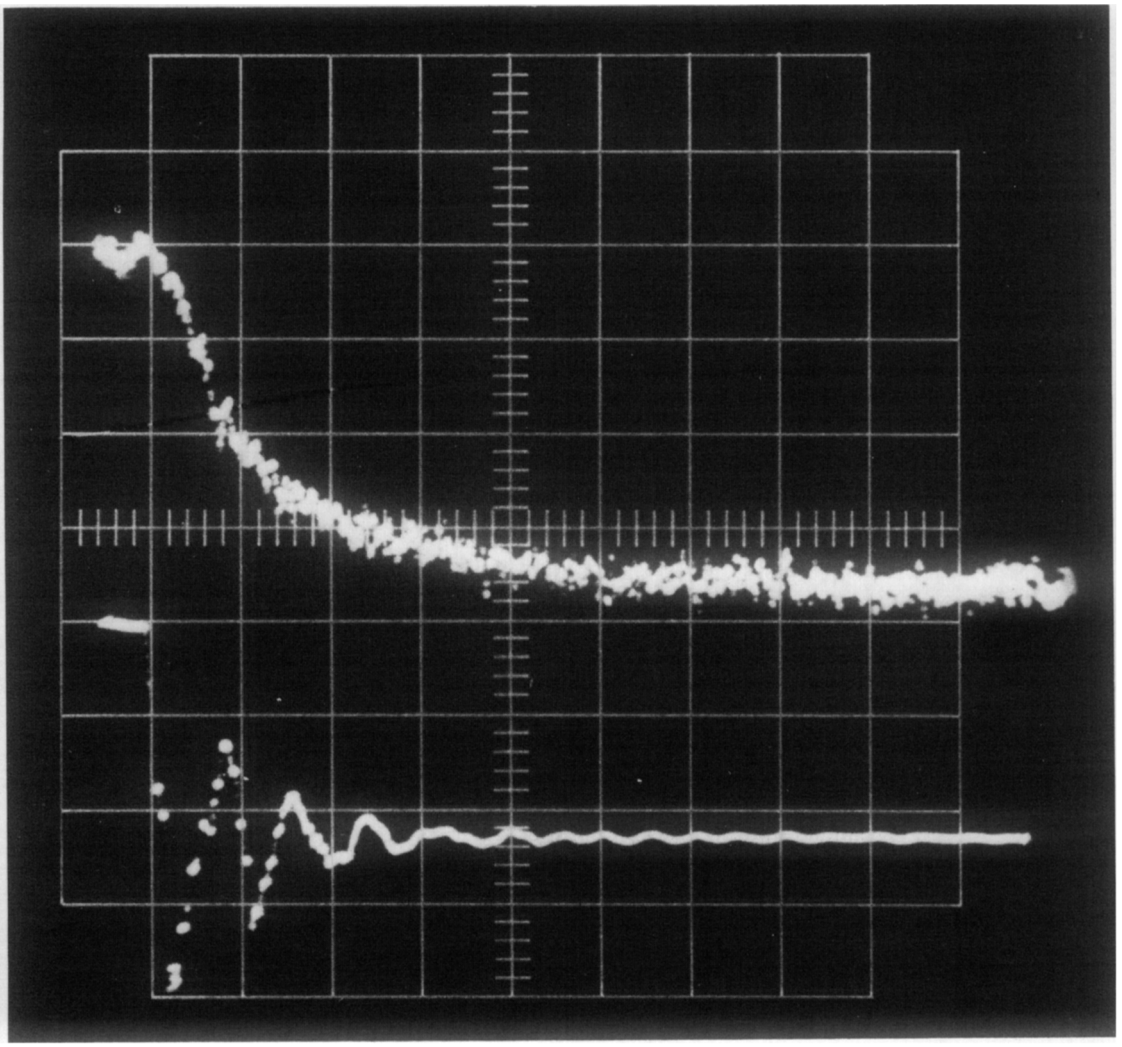

Figure 1. Oscilloscope trace obtained by the fluorescence stopped-flow method for the reaction between DNA-9-aminoacridine complex and polylysine. [9-aminoacridine] = 5 x 10^{-5} M, [DNA phosphate] = 1.5 x 10^{-4} M, [lysine residue of polylysine] = 4.5 x 10^{-4} M (P/D = 3 and P/L = 1/3). Excitation at 400 nm. Fluorescence emission was observed through a cut-off filter transmitting above 430 nm. The upper trace shows the change in fluorescence intensity (increasing downwards): 0.01 volt per major vertical division. The lower trace shows the flow velocity: 9 ml/sec per major vertical division. Horizontal scale: 2 msec per major division.

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ular process (too fast to be observed in the present experiment) is followed by a slow unimolecular one (7).

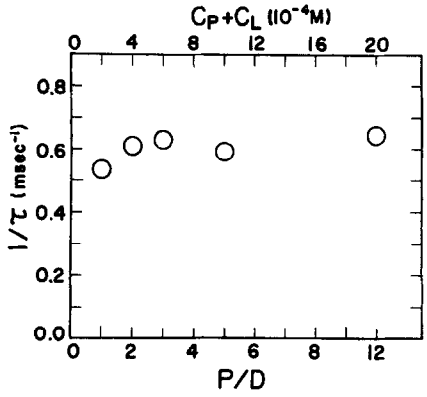

Figure 2. Dependence of the reciprocal relaxation time $1/\tau$ on the P/D ratio and on $(C_P) + (C_L)$. (C_P) and (C_L) are the concentrations of DNA phosphate and lysine residue of polylysine, respectively. The dye concentration and P/L ratio were fixed at 5×10^{-5} M and 1/3, respectively.

A simplest plausible mechanism of the reaction would thus be as follows:

$$\text{DNA-dye} + \text{pLys} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{DNA-dye-pLys} \underset{k_4}{\overset{k_3}{\rightleftharpoons}} \text{DNA-pLys} + \text{dye} \qquad (1)$$

Process I (fast) Process II (slow)

where pLys stands for polylysine. In Process I (a rapid pre-equilibrium), polylysine binds rapidly and randomly to DNA probably through nonspecific electrostatic interaction. A part of bound dye molecules may be expelled in this stage, which is too fast to be observed. In Process II, the randomly bound polylysine twists around DNA to form a stoichiometric tightly bound one-to-one complex between the negatively charged phosphate group and the positively charged ε-amino group, during which the bound (probably intercalated) dye molecules are released from DNA and the increase in fluorescence intensity is observed.

For a dye to be a good probe, the DNA-dye interaction should be sufficiently faster than the DNA-polylysine interaction, irrespective of the binding mode of the dye (intercalation or outside binding (8)). A separate experiment has shown that the interaction

between DNA and 9-aminoacridine is actually too fast to be observed by the stopped-flow method (unpublished results). On the other hand, it is known that the binding mode of a dye depends critically on the P/D ratio. However, the result of Figure 2 that the observed rate is independent of the P/D ratio suggests that the rate is not dependent on the bound state of dye, but is determined essentially by the interaction of DNA with polylysine. Therefore, 9-aminoacridine may be said to be a good probe for studying the interaction between DNA and basic polypeptides.

Since Process I is much faster than Process II, two relaxation times τ_1 and τ_2 ($\tau_1 << \tau_2$) obtained from the above reaction scheme are given by

$$1/\tau_1 = k_1(\bar{C}_P + \bar{C}_L) + k_2 \tag{2}$$

$$1/\tau_2 = k_4 + \frac{k_3(\bar{C}_P + \bar{C}_L)}{K_{21} + (\bar{C}_P + \bar{C}_L)} \tag{3}$$

where $\bar{C}_P$ and $\bar{C}_L$ denote the equilibrium concentrations of DNA phosphate and lysine residue of polylysine, and $K_{21} = k_2/k_1$ (the dissociation constant for Process I) (7). Since Process I is very fast, τ_1 could not be obtained in the present study. From the result shown in Figure 2, and referring to Eq. 3, $(k_3 + k_4)$ (which is equal to the saturation value of $1/\tau_2$) is obtained to be about 600 sec^{-1} and K_{21} is estimated to be less than 10^{-4} M.

The present study has shown the usefulness of 9-aminoacridine as a fluorescent probe in the kinetic study of DNA-polylysine interaction. Further studies are now in progress, including measurements at lower reactant concentrations, the examination of the dependence of the relaxation time on the degree of polymerization of polylysine, and the use of other fluorescent dyes.

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